Tracheary Elements in Ferns: New Techniques, Observations, and Concepts

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ABSTRACT.—Longisections of xylem were studied with scanning electron microscopy (SEM) for roots of *Angiopteris*, upright axes of *Psilotum*, and rhizomes of eight species of leptosporangiate ferns of diverse habits and varied ecological preferences. In contrast to earlier studies using macerations, razor-blade sections of fixed material from living plants were prepared. All materials studied showed porose or reticulate pit membranes present on presumptive end walls of tracheids. Contrasting non-porose pits were observed on lateral walls of some tracheids. Tracheid to parenchyma pit pairs may have porose pit membranes on the tracheid side and nonporose pit membranes on the parenchyma side; thus degree of porosity in a section can represent the degree to which one primary wall or the other is pared away. Reticulate pit membranes on tracheary element end walls are evidently widespread in ferns. Such cells should not be considered vessel elements, although the reticulate pit membranes suggest a degree of transition toward the membrane-free perforations of typical vessels. True vessels (pit membranes absent in perforations) do occur in roots in a limited number of fern genera. The preparation methods of the present study produced results freer from artifacts than did macerations, and interpretations must be altered accordingly. Reports of lateral, multiple, and interrupted perforation plates in ferns are probably the result of loss of pit membranes due to the oxidative action of maceration and should be rejected. Likewise, "pit dimorphism" (alternately wide and narrow pits) and "striate" (corrugated) pit membranes in ferns represent artifacts. True vessel elements in ferns probably always have secondary wall architecture of end walls different from that of lateral walls.

KEY WORDS.—conductive tissue, scanning electron microscopy, tracheids, ultrastructure, xylem

Progress in study of the nature of tracheary elements in ferns has been gradual, limited by techniques and technology. With light microscopy and macerations, vessels were early reported in *Pteridium aquilinum* (L.) Kuhn (Russow, 1873; Bliss, 1939) and *Nephrodium filix-mas* (L.) Schott (Russow, 1873). Gwynne-Vaughan's (1908) reports that vessels occurred in a number of other ferns have been discounted (Bancroft, 1911). Light microscope studies of macerations revealed distinctive perforation plates in roots of *Marsilea* (White, 1961; Loyal and Singh, 1978). White (1962) reported possible vessels in *Astrolepis, Phlebodium, Polystichum,* and *Woodsia.* The report of possible vessels in these four genera by White (1962) was based on the presence of tracheary element end walls that were perceptibly different from lateral walls, an appearance which in turn is based on differences between end walls and lateral walls in secondary wall architecture.

Because we had access to a scanning electron microscope (SEM), we endeavored to confirm White's (1962) reports. In our studies of xylem of *Woodsia obtusa* (Spr.) Torr. (Carlquist *et al.,* 1997) and *Phlebodium* and *Polystichum* (Schneider and Carlquist, 1997), we obtained similar results. In the case of *Woodsia obtusa,* we used SEM study of sections prepared by paraffin sectioning techniques. Excessive fracturing of tracheid walls by

paraffin sectioning in *Pteridium* (Carlquist and Schneider, 1997a) discouraged us from relying on paraffin sectioning, however, and in subsequent studies, we employed macerations studied by SEM.

We confirmed with SEM that tracheary elements with end walls differing from lateral walls in secondary wall architecture also differ by lacking pit membranes in end walls but not in lateral wall pits. This was reported in *Pteridium* (Carlquist and Schneider, 1997a), *Astrolepis* (Carlquist and Schneider, 1997b), *Woodsia ilvensis* (L.) R. Br. (Carlquist and Schneider, 1998a), *W. scopulina* Eat. (Schneider and Carlquist, 1998a), and *Marsilea* (Schneider and Carlquist, 2000b). In these studies, as in those done during this period on vessels of Araceae (Carlquist and Schneider, 1998b; Schneider and Carlquist, 1998b), we thought that macerations provided reasonably reliable material for establishing pit membrane presence or absence. All of the four genera listed above were regarded as having vessels.

In subsequent studies on fern tracheary elements done with macerations, we therefore applied similar interpretive criteria (Carlquist and Schneider, 1998c, 1999, 2000a, 2000b, 2000c, 2001; Carlquist, Schneider *et al.*, 1999, 2000; Schneider and Carlquist, 1998c, 1998d, 1999a, 1999b, 1999c, 2000a); the fern groups studied in these respective papers lack differences between end walls and lateral walls in secondary wall architecture. We observed porose pit membranes, which at that time we considered indicative of vessel presence, on pits of tracheids of all of these ferns. We also observed absence of pit membranes. Since those studies, we became concerned that some artifacts may have resulted from the maceration process. We resolved to reinvestigate selected ferns using fixation with aqueous ethanol solutions, sectioning by hand with razor blades, and drying in air. These methods had provided reliable preservation of pit membranes in tracheary elements for such workers as Meylan and Butterfield (1978) and Sano (2005).

MATERIAL AND METHODS

All materials studied were from rhizomes (stems) of ferns except for *Angiopteris*, in which roots were studied, and *Psilotum*, in which subaerial portions of upright axes were selected. Roots of the ferns available to us other than *Angiopteris* were too slender to be readily sectioned by hand. Suitable portions were fixed in aqueous 70% ethanol. Sections 1–2 mm thick were cut by hand using single-edged razor blades. These sections have the advantage of withstanding the sectioning process better than thinner sections, and our observations with SEM confirmed that delicate primary walls sectioned well and were not damaged. No more than six or eight sections were cut with a given razor blade, because sharpness deteriorates rapidly. Sections were placed between glass slides with gentle pressure applied in order to prevent curling during drying, and were air-dried on a warming table. Dried sections were mounted on aluminum stubs, sputter coated with gold, and examined with a Hitachi 2600N SEM.

Cultivated material from commercial sources was used for the study of *Blechnum brasiliense* Desv., *Cyathea cooperi* (F. Muell.) Domin, *Davallia*

fejeensis Hook., *Pellaea falcata* (R. Br.) Fee, *Platycerium bifurcatum* (Cav.) C. Chr. The specimen of *Angiopteris evecta* (G. Forst.) Hoffm. was cultivated in the tropical greenhouse of the University of California, Santa Barbara. *Psilotum nudum* (L.) P. Beauv. is a common weed in the horticultural complex at that institution. *Polypodium californicum* Kaulf. has become naturalized in the Santa Barbara Botanic Garden. *Cyrtomium falcatum* Presl is adventive in gardens of Santa Barbara. The specimen of *Woodsia obtusa* (Spr.) Torr. was collected in the wild by George Yatskievich and George Taylor along a roadcut in Irons Co., Missouri (for details, see Carlquist *et al.*, 1997). Herbarium specimens (collection numbers by Sherwin Carlquist) of ferns used in this study have been deposited in the herbarium of the Santa Barbara Botanic Garden (SBBG).

RESULTS

Illustrations and descriptions are presented for the "polypod" ferns (Pryer *et al.*, 2004) first, followed by Cyatheaceae, then the eusporangiate ferns.

Blechnum brasiliense (Figs. 1–6).—Longisections of tracheids show a reticulate pattern in some pit membranes (Fig. 1). Tearing of some of the strands in various patterns is readily apparent (Figs. 1, 4, 5), but the reticulate membranes are remarkably intact despite sectioning and handling procedures. Porosities or spaces in the pit membrane reticulum are most readily visible in areas superimposed above pit apertures, because of the greater contrast with a dark background at such locations (Figs. 1, 2, 5). However, the reticulate nature of the pit membrane can also be seen in front of the pit border, and is not limited to the central portion of the pit membrane (Figs. 1–6). Where pit membranes are less porose (e.g., Fig. 1, upper right; Fig. 4, top; Fig. 5, right), the double thickness of the pit membrane of the adjacent cells may be represented on account of the varied degrees to which pit membranes are shaved away in the sectioning process. The more porose pit membrane portion is that of a tracheid; the nonporose pit membrane may be that of a parenchyma cell. The more clearly reticulate patterns may be the primary wall of only one cell in face view. However, the oblique view of Fig. 6 is very valuable in showing that porose membranes occur between adjacent tracheids, where pit membranes of both of the adjacent tracheids must be present (the two seem fused together and not as separate entities in Fig. 6). The fact that the sectioning process is not highly destructive is revealed by the presence of portions of pit membranes so close to the cut surface (Fig. 6). In tracheid pit membranes where no porosities are visible (Fig. 3, upper left), the pit represented is very likely not from a tracheid end wall.

Platycerium bifurcatum (Figs. 7–11).—The longisections of stem tracheary elements show variously porose or reticulate pit membranes. The reticulate nature of pit membranes is clearly evident in Figs. 7, 9, and 10. In some pit membranes, small holes are present (Fig. 8). The reticulum, if present, characterizes the entire pit membrane, as shown in Figs. 9–10). Where the reticulate nature of a pit membrane is not visible over a pit border, as in Fig. 8, reticules in the pit membrane may not be observable because of lack of contrast
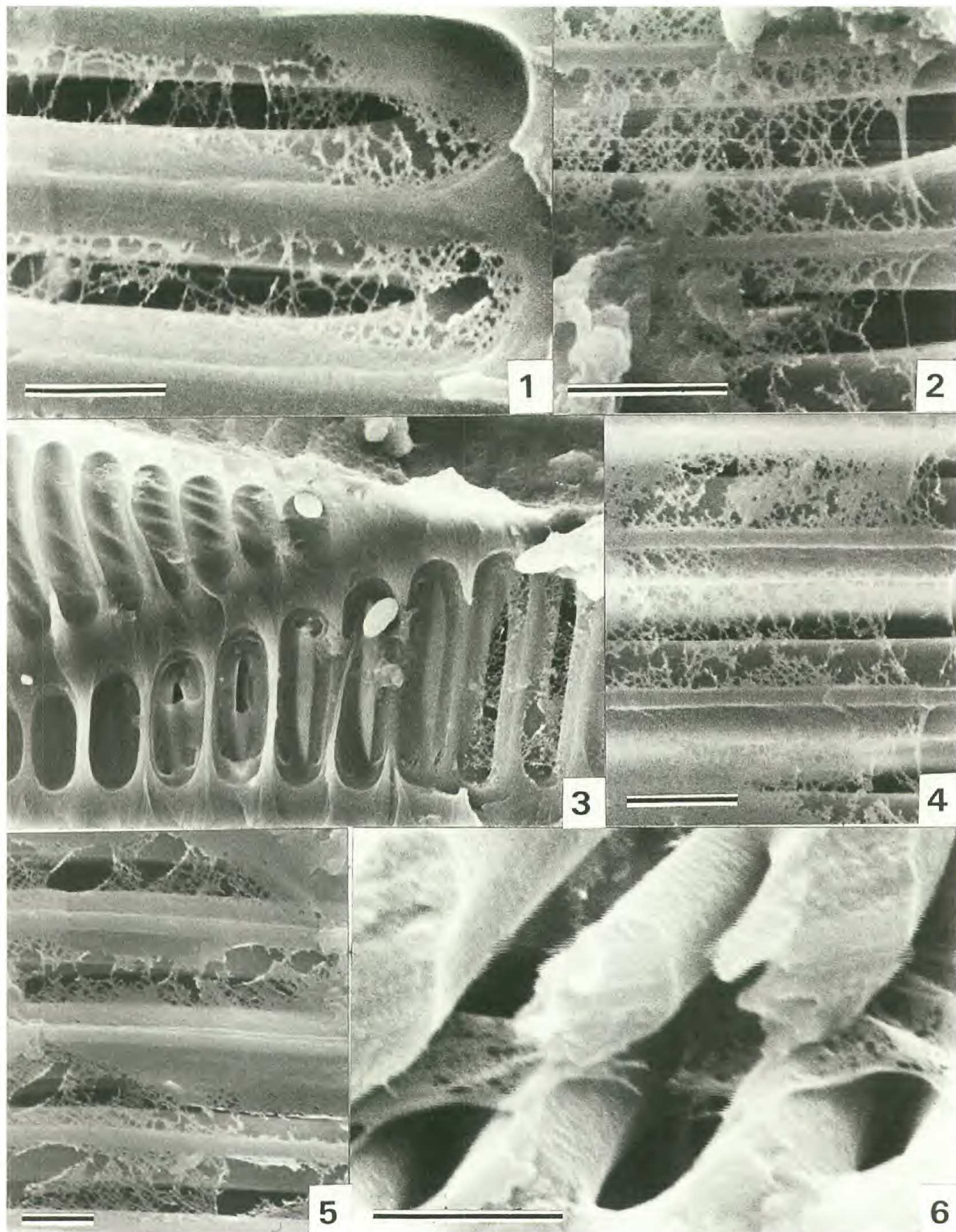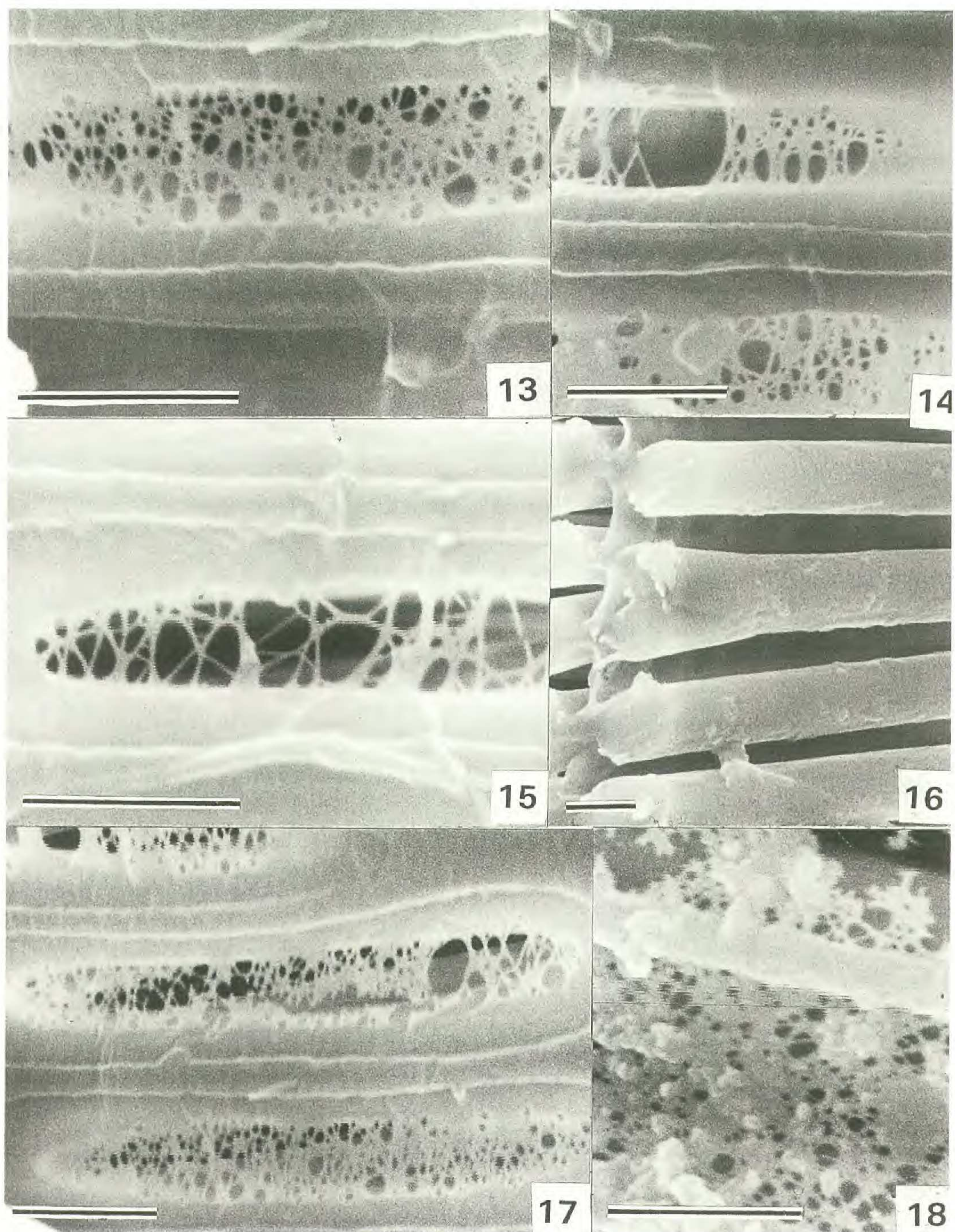
FIGS. 1–6. SEM micrographs of longisections of tracheids of stems of *Blechnum brasiliense*. 1) Portions of two end-wall pits, showing reticulum that becomes finer toward the lateral ends (right). 2) Portions of two end-wall pits, the reticulate pit membranes of which overlie both pit apertures and pit cavities. 3) Portions of two facets; the facet at upper left shows nonporose pit membranes; in the pit membrane at lower right, reticulate pit membranes are illustrated only in the two pits at right, which have experienced more primary wall removal by the sectioning process. 4) Portions of three pit membranes, illustrating a range in pore sizes. 5) Portions of four pit membranes; tearing of pit membranes is distinguishable from the natural reticulum. 6) Oblique view of portions of pits from a cut edge of two adjacent tracheids; the pit membranes between the two tracheids are porose. Scale for all figures = 2 µm.
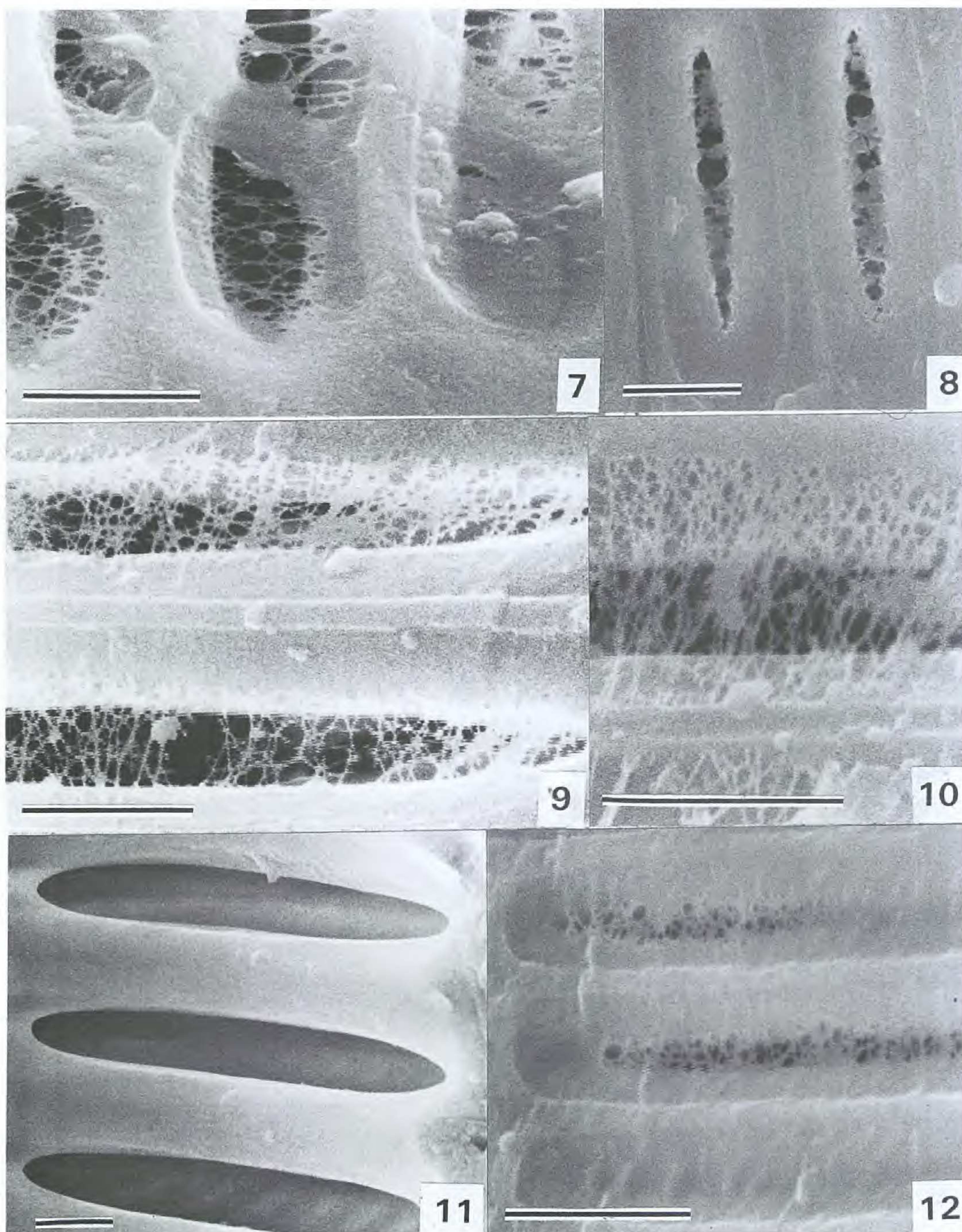
FIGS. 7–12. SEM micrographs of longisections of tracheids of rhizomes of *Platycerium bifurcatum* (Figs. 7–11) and *Pellaea falcata* (Fig. 12). 7) Portions of pit membranes in which prominent reticules are present, although a nonporose membrane is also seen (right). 8) Pit membranes with rather large holes, some of which might represent a degree of artifact formation. 9) Portions of two pit membranes, showing the nature of the reticulum, relatively free from artifacts. 10) Portion of a pit membrane that shows a reticulate nature overlying both pit aperture and pit border. 11) Nonporose pits of a lateral wall, as seen from the inside of a tracheid. 12) Portions of pit membranes that exhibit porose and nonporose areas. Scales = 2 µm.

with the relatively bright underlying pit border, or else the reticule may have collapsed onto the pit border and fused with it as a result of processing. Drying of gel components of the cell wall may account for such pit membrane displacement and adherence. The size of pores in the reticules varies, often appearing to be larger in the center of the membrane, smaller at the margins

Figs. 13–18. SEM micrographs of longisections of tracheids of rhizomes of *Woodsia obtusa* (Figs. 13–17) and *Cyathea cooperi* (Fig. 18). 13) A pit membrane apparently free from artifact formation. 14) Portions of two pit membranes, showing a range in size of pores. 15) Portion of a pit membrane with relatively large holes in the reticulum. 16) A lateral wall between two adjacent tracheids, cut ends of walls at left. 17) Portions of pit membranes, with a rip in one (center), the others apparently intact. 18) Pit membrane portions showing a range in pore size. Scales = 2 μm.

(Figs. 7–10). The lateral wall pitting of tracheary elements lacks any evidence of porosities (Fig. 11).

*Pellaea falcata (*Fig. 12*).*—Porose pit membranes were observed on some tracheid walls (Fig. 12). The pores probably occur throughout a given pit membrane, but are less obvious where they overlie a pit border.

*Woodsia obtusa (*Fig. 13–17*).*—Pit membranes in tracheid end walls contain notably large holes (Figs. 14, 15), although smaller pores are visible also

(Figs. 13, 14, 17). We have no reason to believe that the holes or pores result from artifact formation, because there is very little indication of fracturing in the nonhydrolyzed remnants (= reticulum) of the pit membranes. We were, however, unable to detect pores in marginal portions of pit membranes overlying pit borders (Figs. 13–15, 17). Lateral walls of tracheids were clearly observed to have pit membranes devoid of pores or holes (Fig. 16).

Cyathea cooperi (Fig. 18).—Although material of this species proved difficult because of the sinuous course of vascular bundles, porose pit membranes were detected on several tracheids (Fig. 18). The pores occur randomly over the entire surface of a pit membrane.

Polypodium californicum (Figs. 19–20).—Despite some fracturing of pit membrane strands, the reticulate nature of pit membranes in this species is evident. There is a tendency toward axial alignment of primary wall strands (Fig. 20). The pit membranes of Fig. 19 have pores generally smaller than those shown in Fig. 20.

Cyrtomium falcatum (Fig. 21).—Our material of this species proved difficult to section because the stems have a hard, fibrous texture. Porose pit membranes were evident in pits of a few tracheids.

Davallia fejeensis (Fig. 22).—Small pores were observed to be characteristic of some tracheid pit membranes in this species, although fracturing of the pit membranes was a common occurrence (Fig. 22).

Angiopteris evecta (Fig. 23).—Tracheid pit membranes in roots of this species have a random distribution of pores of various sizes.

Psilotum nudum (Fig. 24).—Minute pores, densely distributed, occur in some pit membranes of the tracheids examined.

Discussion

Our studies using fixation with aqueous ethanol solutions, sectioning by hand with razor blades, and drying in air provided results different from those obtained with macerations. The appearances we report as porosities, reticulate pit membranes, and threadlike pit membrane remnants here are consistent within the specimens studied. Those appearances coexist on specimens with large numbers of pit membranes in which no such structures occur, and the porose to threadlike pit membranes consistently occur in central areas of end walls: the upper and lower tips of end wall surfaces show transitions to nonporose pit membranes. We know of no rigorous comparisons that show that the above methods to be any less reliable than use of methanol or critical point drying in minimizing artifact formation in pit membranes. Cracking in pit membranes, an artifact which probably results from handling as well as to some extent from drying, can easily be differentiated from porose to threadlike pit membrane appearances. We have observed in real time that prolonged exposure to beam current at high magnification can result in fracturing and peeling away of pit membranes, and in production of corrugated pit membrane surfaces. Such appearances are easily recognizable as artifacts and are excluded from our descriptions.
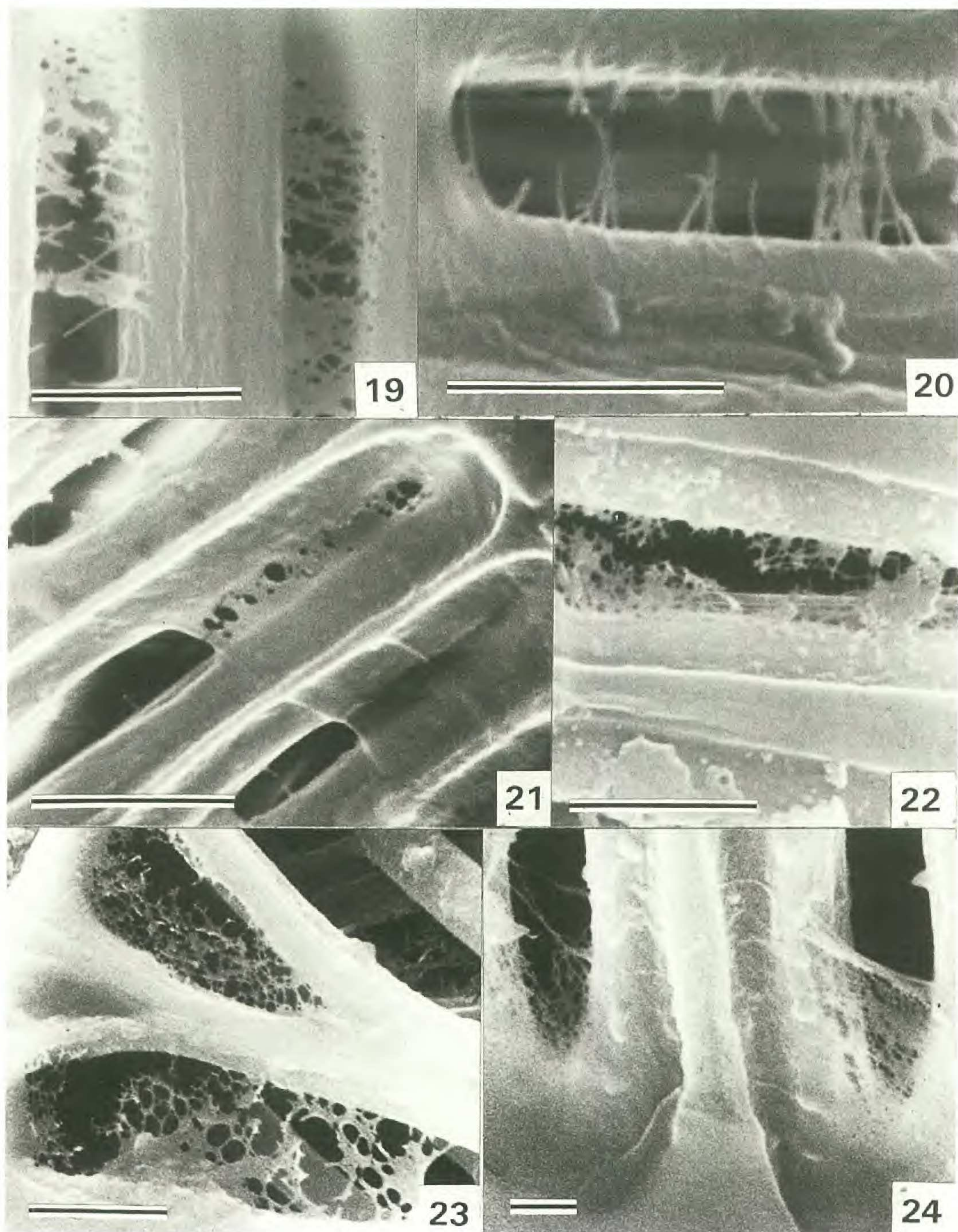
FIGS. 19–24. SEM micrographs of pit membranes from fern tracheids. Figs. 19–22. Longisections from rhizomes. Figs. 19–20. *Polypodium californicum.* 19) Portions of two adjacent pit membranes with different degrees of porousness. 20) Pit membrane remnants tend to be strands oriented in an axial direction. 21) Portions of pits, *Cyrtomium falcatum*; pit membranes present above center, but absent below, apparently because of sectioning. 22) Portion of pit membrane, *Davallia fejeensis*, showing a pit membrane with small pores; the rather extensive tearing is an artifact. 23) *Angiopteris evecta,* pit membranes from longisection of a root, showing reticula broken by some tearing. 24) *Psilotum nudum,* portions of two finely porose pit membranes from a longisection of the subaerial portion of an upright axis. Scales = 2 μm.

Our newer results have produced, in our opinion, reliable images of the nature of pit membranes in tracheary elements in ferns, and therefore we are offering new conclusions here, together with reassessments of our earlier studies. Broader issues, such as the terminology of tracheary elements in the

light of ultrastructural knowledge, and the possible functions of porose and reticulate pit membranes, come into question and are therefore discussed here.

Distribution of porose pit membranes in fern tracheids.—The present study could have been extended indefinitely to obtain more images of porose or reticulate pit membranes in fern tracheids, because this feature was observed in all of the ferns studied. The selection was not biased in favor of particular habit types or habitat preferences, so there appears no relationship, in rhizome tracheids at least, between the occurrence of such membranes and plant form or ecology. Epiphytes, tree ferns, rhizomatous ferns, and rosette ferns were all represented. Nonporose pit membranes were observed in lateral wall pits of tracheids or in tracheid-to-parenchyma pits wherever material of xylem was relatively abundant and we could make comparative observations. Our earlier SEM studies of macerated tracheids showed that tracheary end walls free of pit membranes occurred in roots of *Astrolepis*, *Marsilea*, *Pteridium*, and *Woodsia*. Discounting reports of vessels in ferns other than these, our work showed finely porose pit membranes in a surprising number of ferns (Carlquist and Schneider, 2001). Our present study confirms that porose and reticulate pit membranes do occur widely in fern tracheids. Our renditions of these delicate structures seem much more accurate in the present study. Oxidative action of the macerating fluid in our earlier studies, we believe, did not create the porous appearances, but its action in removing portions of pit membranes is now evident. The picture that emerges is that reticulate to porose pit membranes are characteristic on end walls of fern tracheids. Variations in these networks occur, and need further exploration.

Defining the vessel element.—In dicotyledons, vessels are defined by having four features (Carlquist and Schneider, 2002a): (1) there are one or more perforations (free of pit membranes) on the end wall; (2) the end wall architecture (perforations) is different from that of the lateral wall (pits); (3) vessel elements are shorter than the imperforate tracheary elements they accompany; (4) vessel elements are wider than the imperforate tracheary elements they accompany. The last two features represent a division of labor between two conductive cells possible in vascular plants with vascular cambia, and aside from dicotyledons, only *Ephedra* and *Gnetum* possess this feature. Thus, only the first two criteria are applicable to ferns and monocotyledons. In ferns, only the genera *Astrolepis*, *Marsilea*, *Pteridium*, and *Woodsia* (see Introduction) satisfy the second criterion by having secondary wall architecture of end walls different from that on lateral walls. Such distinctive end walls possibly may occur in a few other genera.

The first criterion, presence or absence of pit membranes on the end walls of tracheary elements, has been judged until the past two decades on the basis of light microscopy. Light microscopy thus is the source of definitions currently used in textbooks. When SEM study is used, one does find genuine absence of pit membranes in end walls (thus perforation plates) of many monocotyledons and dicotyledons, but in some putatively primitive families of both, pit membrane remnants do occur (e.g., Carlquist, 1992; Carlquist and Schneider, 2002b, Schneider and Carlquist, 2003). In fact, within the genus *Illicium*,

various degrees of pit membrane occurrence occur. In *I. anisatum* L. and *I. floridanum* J. Ellis, one could designate vessels present or absent in terms of pit membrane presence in end walls: pit membranes may be nonporose, porose, strandlike, and reticulate. In *Illicium*, the three criteria of vesselhood other than pit membrane absence in end walls are satisfied.

In ferns other than *Astrolepis*, *Marsilea*, *Pteridium*, and *Woodsia*, degree of pit membrane presence is the only criterion by which vessel presence might be claimed. If one says that possession of reticulate pit membranes, as in *Blechnum* and *Platycerium*, could constitute a criterion, one could conceivably develop a criterion that hydrolysis of more than 50% of the pit membrane would result in formation of vessels, and thus *Blechnum* and *Platycerium* might be said to have vessels. Clearly, ultrastructure erases any usable boundary between tracheids and vessel elements in a number of cases. The most sensible solution seems to be to call attention to intermediate conditions when they occur. The inherent interest is not in the definition, but in the evolutionary and physiological significance of this phenomenon.

Possible functional significance of tracheid ultrastructure.—Given the widespread occurrence of weblike or porose pit membranes or pit membrane remnants in ends walls of fern tracheids, the probability exists that this structural mode represents an adaptive system. Reticulate or porose pit membranes would represent an intermediate stage on the way to attainment of vessels. Intermediate stages, however, are often labile, whereas the tracheid end-wall pit membranes of ferns are not. One can compare the condition in ferns to the similar differentiation in end walls of *Tetracentron* tracheids (see Carlquist, 1992). Porosities in the margos of coniferous tracheid pit membranes also bear comparison. The margo pores illustrated for Podocarpaceae by Meylan and Butterfield (1978) are in the same size range as the pores or reticular spaces we figure for ferns. In coniferous tracheids, the margo pores may increase conductive rate (Zimmermann, 1983). The larger pores of a coniferous margo can allow air passage under some conditions, a process shut down by aspiration of the pit as the torus is forced against the pit border. The nonhydrolyzed cellulosic strands of the conifer pit margo permit the torus to function in pit aspiration; the openings among the strands are relatively large. The margo pores in conifers that are likely to transmit air are often about 1 μm in diameter (Zimmermann, 1983), whereas the pores in fern tracheid pit membranes are much smaller, as our illustrations show. Fern tracheids do not have tori, and thus pores as large as those of a conifer pit margo would risk embolism passage. The adaptive significance of the porose or reticular pit membranes in end walls of fern tracheids thus appears to be enhancement of conductive rates without increase in the risk of embolism. Understanding of the physiological action of fern tracheids has lagged behind understanding of the relationship between ultrastructure and physiology in the case of conifer woods, very likely because of the economic value of conifer woods. Porose pit membranes certainly do occur between adjacent tracheids of ferns, as shown in Fig. 6. The possibility remains that where nonporose membranes exist on the

same facet as porose membranes (e.g., Figs. 3, 7), pores may only partially perforate the pair of adherent primary walls between tracheids.

In those ferns that do have vessels in the roots, one can cite ecological correlations. A number of monocotyledons have vessels in the root only. These monocotyledons exist in wet areas that may dry seasonally. Under such conditions, rapidity of conduction in roots is of selective value, whereas the lack of vessels in stems of those monocotyledons may inhibit spread of embolisms into stems, which are the perennating organs of monocotyledons (Carlquist, 1975). This statement would apply to *Marsilea*, which often grows in ponds of limited duration. *Pteridium* and *Woodsia* grow in habitats where temperatures and water availability show strong fluctuation.

Artifacts; reinterpretation of earlier data.—Our earlier studies on fern xylem were based on macerations prepared with Jeffrey's fluid. Macerations of dicotyledonous woods and even of xylem of monocotyledonous roots and stems (Carlquist and Schneider, 1998b; Schneider and Carlquist, 1998b) tend to leave pit membranes in lateral walls of vessel elements intact. We therefore assumed that these methods would result in similar results with fern xylem. That assumption proved faulty because fern roots and stems are refractory when treated with Jeffrey's fluid. Successful macerations require prolonged treatment, apparently because the xylem is associated with thick fibrous sheaths. Although secondary wall architecture was not damaged by prolonged maceration, we believe that the integrity of primary walls was lost to various degrees by prolonged oxidative treatment. We therefore turned to nonoxidative methods, such as those used by Sano (2005) in ultrastructural studies of cell walls. The preparation methods of Meylan and Butterfield (1978) were similar, where study with SEM was concerned.

We believe that revisions in our earlier reports are therefore required. Certainly presence of porous pit membranes, reported in our earlier studies, has been confirmed in the present study, although with better preservation and imaging. However, the reports of multiple perforation plates, lateral perforation plates, and intermittent perforations in fern tracheids should be regarded as erroneous. Reports of perforation plates lacking any pit membrane remnants in such genera as *Microgramma* and *Vandenboschia* (Carlquist and Schneider, 2001) are probably the result of excessive maceration. All reports of vessels in genera other than *Astrolepis*, *Marsilea*, *Pteridium* and *Woodsia* should be regarded as incorrect or possibly incorrect. Likewise, reports of pit dimorphism (alternately wide and narrow pits or perforations) should be regarded as results of artifact formation.

Air drying of sections, employed by Meylan and Butterfield (1978) and by Sano (2005) as well as in our present study, does not induce any serious artifact formation and preserves reticulate or porose pit membranes, the "microfibrillar webs" of Meylan and Butterfield (1978). We compared the results of varied settings for accelerating voltage and beam current in our present study and did not find that differences in these settings produced artifacts. Artifacts, such as tearing or cracking of pit membranes, are readily recognized as stress-induced phenomena. Pit membranes that are "striated"

(minutely corrugated) occurred both in our earlier studies and in the present study (Fig. 3, upper left) and very likely result from kinds of stress, such as heating by the SEM electron beam. Such stress artifacts are easily recognizable as different from the reticulum of pit membranes that results from natural hydrolysis of the cell wall. In our present study, we are aware that sections that show both reticulate and nonreticulate pit membranes may reveal differences in layers of the pit membrane (e.g., Fig. 12), although pores can be observed to perforate the entire thickness of the primary wall (as in Fig. 6). Thus, sections must be studied with care and with regard to context. On one side of a pit pair, a pit membrane may be reticulate, whereas the pit membrane of the adjacent cell may be nonporose. Portions of both pit membranes can be exposed by our sectioning technique. We hope that our work will be helpful to those dealing with xylem ultrastructure by pointing the way toward reliable methodology.

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